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A simulated heat wave shortens the telomere length and lifespan of a desert lizard

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ABSTRACT

Understanding how organisms respond to warming contributes important information to the conservation of biodiversity that is threatened by climate warming. Here, we conducted experiments on a desert agama (*Phrynocephalus przewalskii*) to test the hypothesis that climate warming (an increase in both mean temperature and heat waves) would induce oxidative stress, shortening telomere length, and thereby decreasing survival. Our results demonstrated that one week of exposure to a simulated heat wave significantly shortened telomere length, and decreased the overwinter survival of lizards, but mean temperature increase did not affect the survival of lizards. However, the antioxidant capacity (anti-oxidative enzyme) was not affected by the warming treatments. Therefore, heat waves might have negative impacts on the desert agama, with shortened telomeres likely causing the lifespan of lizards to decrease under climate warming.

1. Introduction

Ongoing climate warming is enhancing the global mean surface temperature and increasing temperature variability, with more hot extremes occurring (i.e., heat wave) (IPCC, 2013; Thornton et al., 2014), which has pervasive and profound impacts on the life of organisms and ecosystems they use (Thomas et al., 2004; Barange et al., 2014). Reptiles are especially vulnerable to climate warming because their behaviour, physiology, and life history are highly dependent on environmental temperature (Deutsch et al., 2008; Sinervo et al., 2010; Huey et al., 2012). A recent study showed that lizards might grow fast, but die young, in the context of climate warming (Bestion et al., 2015). However, the proximate mechanisms underlying the shortened lifespan of lizards have not been explicitly revealed. One such proximate mechanism might involve the disturbance of balance between the oxidation and antioxidant defence systems that causes oxidative stress. For instance, heat exposure induces oxidative stress that shortens the length of telomeres, which are complex DNA-protein caps of eukaryotic chromosomes that function to maintain genome integrity (von Zglinicki, 2002), shortened telomere length might be associated with ageing and therefore the survival and lifespan of animals, although this relationship is not uniform across taxa (Finkel and Holbrook, 2000; Monaghan, 2010; Banh et al., 2016).

Global drylands have experienced, and will continue to face, much more severe increases in temperature than humid areas (Huang et al., 2017). Lizards that inhabit drylands are especially sensitive to climate warming, due to sparse vegetation cover, which provides limited opportunities for thermoregulation to cool down their bodies (Kearney et al., 2009). In this study, we conducted a warming experiment to determine how an increase in mean temperature and heat waves influences the survival rate, oxidative stress, and telomere length of the desert toad-headed agama, *Phrynocephalus przewalskii*. In particular, we aimed to test the hypothesis that an increase in mean temperature and heat waves induces oxidative stress that might shorten telomere length and cause the survival (and, therefore, lifespan) of lizards to decrease.

2. Materials and methods

2.1. Study species

The desert toad-headed agama (*Phrynocephalus przewalskii*) is a small lizard species [44–56 mm adult snout-vent length (SVL)] that

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inhabits arid and semi-arid regions (Zhao et al., 1999). The preferred body temperature, critical thermal maximum (CT_{max}), and critical thermal minimum (CT_{min}) of this species are 36.6 °C, 47.1 °C and 1.3 °C, respectively (Qu et al., 2011; Li et al., 2017). In summer, the operative temperatures (T_es) differ among microhabitats, ranging from 28.9 ± 1.3 °C in full shade to 41.2 ± 1.3 °C in full sun. Furthermore, field body temperatures fluctuate with the time of day, with a mean body temperature of 38.1 ± 0.2 °C during the daytime period from 09:00–17:00 (Li et al., 2017).

2.2. Experimental design and thermal treatments

To simulate the thermal environment of lizards with greater precision and to design the thermal regimes for the warming experiments, we determined the field body temperature of lizards in August 2012 and 2013. We captured adult *P. przewalskii* by hand at our field site from 08:00 to 18:00, and the body temperature of the captured lizards was measured immediately (± 0.1 °C) by inserting the probe of the UT325 electronic thermal meter (Shenzhen Meter Instruments, Shenzhen, China) into the cloaca (about 5 mm). The body temperature of the lizards at night (from 19:00 to 07:00) was estimated from the ambient temperature of the burrows where they hide at night. The burrow temperatures were recorded by iButtons (DS1921, MAXIM Integrated Products Ltd., USA). Accordingly, the daily body temperature was 32 ± 8 °C (range: 24.8–39.5 °C; Fig. A.1). This information formed the basis of the temperature treatment of the control group in this study. In addition, by the end of the current century, the global mean surface temperature is predicted to increase 0.3–4.8 °C, depending on various global emissions scenarios (IPCC, 2013).

Based on this information, we designed three thermal regimes for the warming experiment: control, warming, and heat wave groups. These regimes simulated the thermal environment experienced by lizards under the current climate, climate warming of 3 °C, and climate warming of 3 °C accompanied by a heat wave of one-week, respectively. The lizards in the control and warming groups were kept at 32 ± 6 °C and 35 ± 6 °C throughout the experiment (14 days), whereas lizards in the heat wave group were primarily maintained at 32 ± 6 °C, and at 38 ± 6 °C for seven days (day 5–11) (Fig. 1). Accordingly, the lizards in the warming and heat wave groups experienced a similar higher (3 °C) temperature (but with different hot temperature extremes) than the lizards in the control group. The experiment was conducted in three programmable incubators (Binder KB 240, Binder GmbH, Tuttlingen, Germany) in which the daily temperatures were set to the regimes of 32 ± 6 °C, 35 ± 6 °C, and 38 ± 6 °C, respectively.

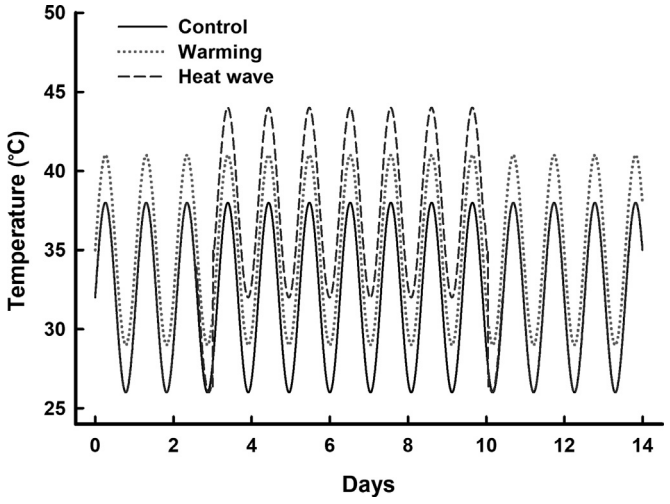

Fig. 1. Three thermal treatments used in the present study. The temperature regime of the control group (solid line) was 32 ± 6 °C, mimicking the daily variation of lizard body temperature in the field. The temperature regime of the warming group (red dotted line) was 35 ± 6 °C, mimicking the warming scenario of a 3 °C future increase in the mean temperature. The temperature regime of the heat wave group (blue dotted line) was 32 ± 6 °C, but with seven days (day 5–11) of 38 ± 6 °C, mimicking the hot extremes that are expected to occur during climate warming.

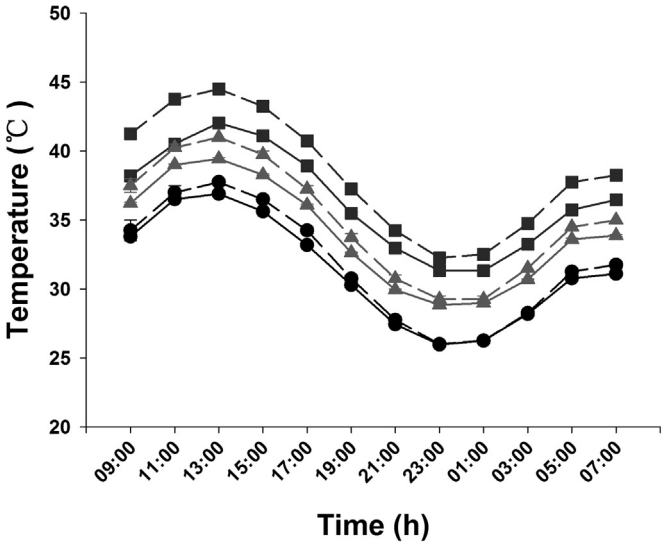

Fig. 2. The diel variation of ambient temperatures and body temperatures of *Phrynocephalus przewalskii* lizards under the three thermal treatments of 32 ± 6 °C (•), 35 ± 6 °C (▲), and 38 ± 6 °C (■). —— body temperature, ——— ambient temperature.

In late September 2015 (post-breeding season), we collected 88 adult *P. przewalskii* (male:female = 49:39) from Jungar Banner, Inner Mongolia, China (40° 12′ N, 111° 07′ E; elevation 1036 m). The lizards were transported to our laboratory in Beijing, where they were kept in three containers (600 × 430 × 340 mm) within a climate controlled room with a temperature of 24 °C and a photoperiod of 14:10 (L:D). To produce a thermal gradient for lizard thermoregulation, a full-spectrum bulb (25 W) was set at one end of all containers to provide heating source from 09:00 to 16:00 every day. For all individuals, snout–vent length (SVL) was measured to 0.01 mm, and body mass (BM) was weighed to 0.001 g by using a vernier caliper (Kanon Instruments, Japan) and an electronic balance (Mettler-Toledo GmbH, Greifensee, Switzerland). After one-week of acclimation, these lizards were allocated randomly to three temperature treatments [control (n = 28, M:F = 16:12), warming (n = 30, M:F = 16:14), and heat wave (n = 30, M:F = 17:13)] to evaluate how experimental warming affects the oxidative stress and survival of lizards. In each treatment, 8–10 lizards were kept in each of three containers (600 × 430 × 340 mm). The bottom of each container was filled with 5 cm-thick sand to mimic the habitat usually occupied by the lizards. The lizards buried themselves in sand for thermoregulation. The photoperiod was 14:10 (L:D). Food (crickets, *Acheta domesticus*, dusted with mixed vitamins and minerals) and water were provided ad libitum. On the fifth day of the warming experiment, we measured the cloacal temperatures of 30 lizards using an electronic thermometer (UNT325, Unitrend electrical limited Liability Company, Shanghai, China). The body temperatures of lizards were 31 ± 0.3 °C, 34 ± 0.3 °C and 36 ± 0.3 °C when they were exposed to thermal regimes of 32 ± 6 °C, 35 ± 6 °C, and 38 ± 6 °C respectively (Fig. 2).

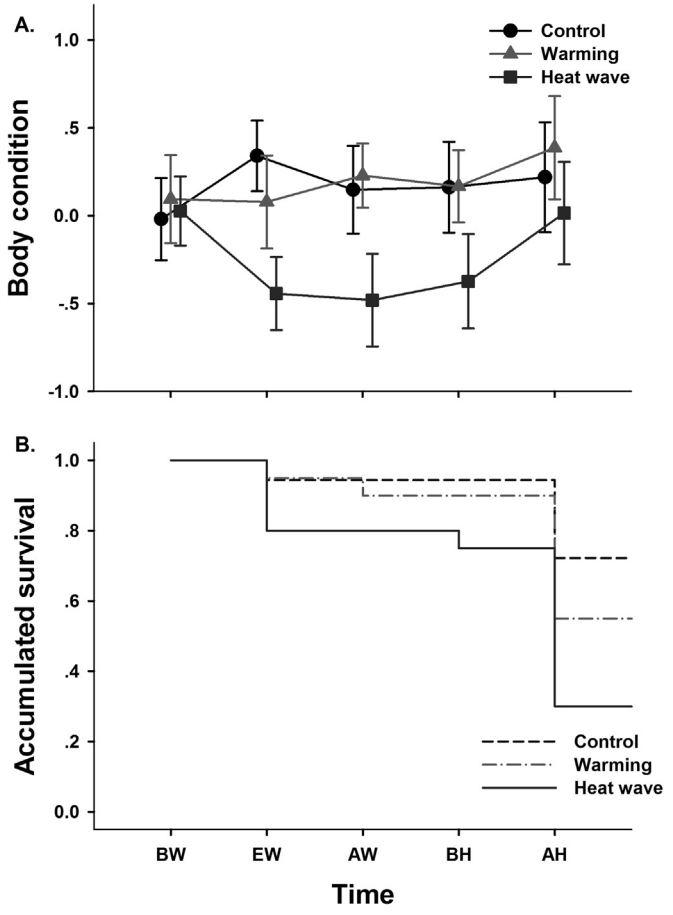

Fig. 3. Body condition (A) and survival rate (B) of *Phrynocephalus przewalskii* in three thermal treatments at the beginning (September 23, 2015) and end (October 6, 2015) of the warming experiment (BW and EW, respectively), one month after the warming experiment (November 6, 2015) (AW), and before (November 23, 2015) and after (March 12, 2016) hibernation (BH and AH, respectively).

2.3. Antioxidant assay and telomere length

Immediately after the warming experiment, some of the lizards (n = 30, M:F = 1:1) were killed with a lethal injection of sodium pentobarbital. Out of all body tissues, the heart is the most prone to reactive oxygen species (ROS) damage because it is an aerobic organ that has high mass-specific oxygen consumption rates (Magni et al., 1994). Therefore, we collected the hearts from the euthanized lizards to measure anti-oxidative enzyme (superoxide dismutase, SOD) and telomere length. The collected hearts were cut into small pieces. After washing the tissue with ice-cold saline to remove residual blood, they were snap-frozen in liquid nitrogen and stored at −80 °C until assay.

To evaluate the antioxidant capacity of the lizards, we measured SOD activity, which is an enzyme whose sole function seems to be the removal of ROS (Finkel and Holbrook, 2000), using specific kits (SOD Assay Kit-WST; Nanjing Jiancheng, Nanjing, China) according to the manufacturer's instructions. One unit of SOD was defined as the amount of enzyme that causes 50% inhibition of the superoxide radical produced by the reaction between xanthine and xanthine oxidase at 37 °C. SOD activity = SOD inhibition % / (1 – SOD inhibition %) unit, in which SOD inhibition % = $(A_{\text{Blank control 1}} - A_{\text{Standard or Sample}})/(A_{\text{Blank control 1}} - A_{\text{Blank control 2}}) \times 100\%$, in which the absorbance (A) was read at 450 nm using a microplate reader.

To determine telomere length, genomic DNA was isolated from the heart using a DNeasy Blood & Tissue Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). DNA quantification was performed using an ND-1000-Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Telomere length was quantified using real-time quantitative PCR developed to measure relative telomere length, which has been previously described where TTAGGG repeat content is expressed as the ratio of telomere repeat copy number (T) to a control single gene copy number (S) (Cawthon, 2002). In our study, the single copy gene 18S rRNA was used as a control. The relative telomere length was calculated following the formula: telomere length = $2^{(-\triangle Ct)}$ where $\triangle C_t = C_t^{\text{Telomere}} - C_t^{18S}$ (Cawthon, 2002), the C_t (cycle threshold) of a DNA sample is the fractional number of PCR cycles to which the sample must be subjected in order to accumulate enough products to cross a set threshold of magnitude of fluorescent signal.

Quantitative PCR was performed using the SYBR Select Master Mix (Takara, Dalian, China) with an ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA). The forward and reverse primers of the telomere were 5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′ and 5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′ (Callicott and Womack, 2006). The control single copy gene of 18S rRNA was amplified using the primers of 18 S rRNA-F (5′- ACTCAACACGGGAAACCTCA-3′) and 18S rRNA-R (5′- AACCAGACAAATCGC TCCAC-3′) (Mallatt and Winchell, 2007). We used 25 ng of DNA per reaction of qPCR for both telomeres and 18S. The total volume was 10 μl (9 μl of master mix + 1 μl of DNA). The master mix contained 2 μl of each primer and 5 μl of Applied Biosystems SYBR Green PCR Master Mix. PCR conditions for telomere and 18S were 5 min at 95 °C, followed by 40 cycles for 15 s at 95 °C, 15 s at 60 °C, and 15 s at 72 °C. Both reactions ended with a dissociation program of 1 min at 95 °C, 30 s at 55 °C, and 30 s at 95 °C.

2.4. Body condition and survival of lizards

The remaining lizards (n = 58) [control (n = 18, M:F = 11:7), warming (n = 20, M:F = 11:9), and heat wave (n = 20, M:F = 12:8)] were raised under the same environmental conditions until Nov 25, 2016; specifically, a temperature of 28 ± 0.3 °C (75 W electronic heating mats beneath containers provided opportunities for behavioural thermoregulation), humidity of 22 ± 0.76%, and a photoperiod of 14:10 (L:D). Food (mealworms and crickets dusted with additional vitamins and minerals) and water were provided daily, ad libitum. Before hibernation, 9 individuals died; the remaining 49 individuals were randomly distributed among six containers (370 × 300 × 130 mm). The hibernation experiment was conducted in programmable incubators (Binder KB 240, Binder GmbH, Tuttlingen, Germany) at 1.5 °C during winter (from November 24 of the first year to March 11 of the next year).

We measured the snout-vent length (SVL), body mass (BM), and mortality of experimental lizards at the beginning (September 23, 2015) and end (October 6, 2015) of the warming experiment, one month after the warming experiment (November 6, 2015), and before (November 23, 2015) and after (March 12, 2016) hibernation. Body condition was quantified using residual scores from the linear regression of $\log_e$-transformed BM to $\log_e$-transformed SVL. The survival rate of lizards was calculated as the percentage of individuals that survived.

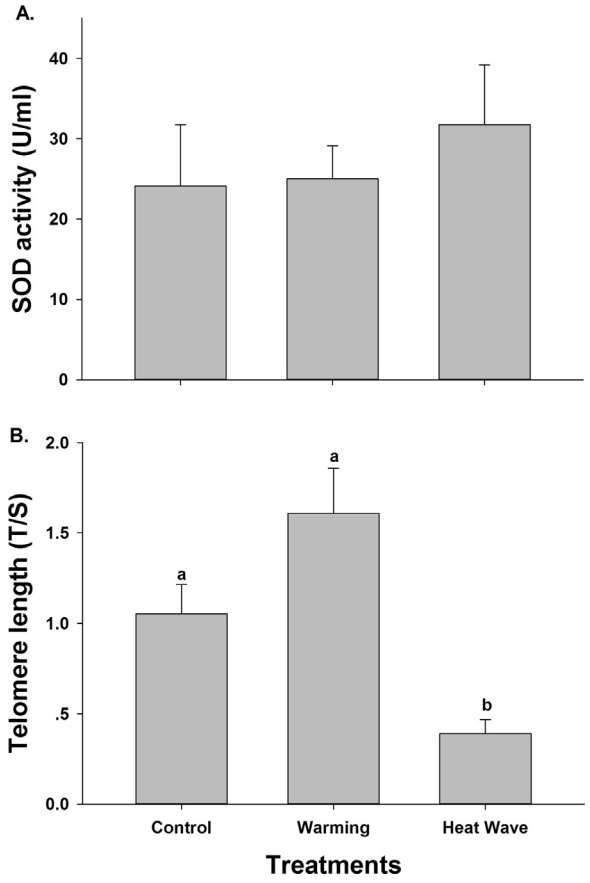

Fig. 4. SOD activity (A) and telomere length (B) of *Phrynocephalus przewalskii* in the control, warming, and heat wave groups. Letters above each bar indicate significantly different groups (ANOVA, $P < 0.05$). SOD: Superoxide dismutase. Values are means ± SE. The sample size for each group was 10.

2.5. Statistical analysis

We tested the normality of distributions and homogeneity of all variances in the data with the Kolmogorov-Smirnov test and Bartlett's test before the analysis. Data were square root transformed to meet the requirement of the parametric analysis when needed. There was no significant sex or sex × temperature effects on any of the variables determined in this study (all $P > 0.05$); therefore, sex was not included as a factor in the subsequent analysis. One-way ANOVAs, with the thermal treatments as fixed factors, were used to detect how the thermal treatments influenced the SVL, body condition, SOD activity, and telomere length of the lizards. We used the Tukey HSD method in the post hoc tests for traits that met the homogeneity of variances assumption; otherwise, we used the Dunnett T3 method. The cox regression analysis was used to compare between-treatment differences in the survival rate of lizards. Data are presented as mean ± S.E., except for mortality. $P < 0.05$ was considered statistically significant. All of the analyses were performed using SPSS ver. 23.0.

3. Results

Neither the body size (SVL) or the body condition (BC) of lizards differ significantly among the control, warming, and heat-wave groups at the beginning of the warming experiment (SVL: $F_{2,55} = 1.360$, $P = 0.265$; BC: $F_{2,55} = 0.137$, $P = 0.872$), at the end of the warming experiment (SVL: $F_{2,50} = 0.271$, $P = 0.763$; BC: $F_{2,50} = 2.860$, $P = 0.067$), one month after the warming experiment (SVL: $F_{2,48} = 0.668$, $P = 0.518$; BC: $F_{2,48} = 2.899$, $P = 0.065$), and before (SVL: $F_{2,46} = 0.178$, $P = 0.837$; BC: $F_{2,46} = 1.642$, $P = 0.205$) and after (SVL: $F_{2,26} = 0.369$, $P = 0.695$; BC: $F_{2,26} = 0.305$, $P = 0.740$) hibernation (Fig. 3A). Of 18, 20 and 20 lizards from control, warming, and heat wave group, respectively, 12, 11 and 6 lizards survived to next spring. The overwinter survival rate of lizards from the heat-wave group was significantly lower than that of lizards from the control group ($\chi^2 = 6.713$, $P = 0.010$), and slightly lower than that of the warming group ($\chi^2 = 3.025$, $P = 0.082$). However, the overwinter survival rate of lizards from the warming group did not differ from the control group ($\chi^2 = 0.174$, $P = 0.676$; Fig. 3B).

SOD activity did not differ among the thermal treatments ($F_{2,27} = 0.401$, $P = 0.672$; Fig. 4A). In contrast, telomere length was significantly shorter for lizards from the heat-wave group compared to the control and warming groups ($F_{2,27} = 16.010$, $P < 0.001$; Fig. 4B).

4. Discussion

Remarkably, our study demonstrated that even a short period (one-week) of exposure to heat wave significantly shortened the telomere length and decreased the overwinter survival of a desert lizard compared with those exposed to thermal environments mimicking the current climate. In contrast, exposure to 3 °C increased mean temperature (the warming group) did not decrease telomere length or survival. Accordingly, our results imply that mean temperature increase accompanied by hot extremes may impose negative impacts on the survival of this desert lizard. The underlying mechanisms could be that the increased ROS and oxidative stress induced by heat wave accelerate telomere shortening that hastens cell senescence, and the replicative senescence of cells could contribute to the death of individuals and therefore the decreased survival rate of a population (von Zglinicki, 2002).

It is noteworthy that natural habitats probably provide lizards more opportunities for thermoregulation to alleviate the impact of heat wave, although our experimental set-up has incorporated important aspects of the natural environment (i.e. sand to burrow for thermoregulation). Despite the buffering effect of behavioural thermoregulation, the desert agama may suffer from heat waves because this species spends over 80% of active time in full sun or filtered sun sites, and has high body temperatures close to critical thermal maximum in summer ((Li et al., 2017) and Fig. A.2). However, mixed results were obtained from previous studies on the biological effect of heat waves. Some studies found that heat waves had a negative effect on organisms (Garrabou et al., 2009; Ma et al., 2015), while others did not (Pipoly et al., 2013; Stahlschmidt et al., 2017). This discrepancy might be due to interspecies differences in thermal safety margins, as well as across-study differences in experimental design. Ectothermic animals from tropical and desert areas have narrower thermal safety margins, and might thus be more sensitive to heat waves than those from temperate zones (Sunday et al., 2014; Li et al., 2017). In addition, between-species differences in body sizes may also account for the discrepancy partially,

because body size does affect heat exchange (e.g. radiation and convection) between an organism and its environments and therefore body temperature and thermoregulation of ectotherms (Campbell and Norman, 1998).

Telomere length is positively related to individual fitness, predicting the longevity and lifetime reproductive success in some, but not all vertebrates examined (Bize et al., 2009; Olsson et al., 2010) (Kotrschal et al., 2007). Accordingly, shortened telomere length induced by heat waves provides a proximate explanation for decreased survival and, thus, the shortened lifespan of lizards under the condition of climate warming (Bestion et al., 2015). However, we did not detect any significant among-treatment differences in the activity of SOD, which is an antioxidant enzyme that defends against ROS. This result was not consistent with the prediction that environmental stress induces the high activity of anti-oxidative enzymes (Finkel and Holbrook, 2000). Further studies are needed to determine whether heat waves induce the activity of anti-oxidative enzymes other than SOD, like catalase and glutathione peroxidase.

In conclusion, our results on shortened telomere length and decreased survival induced by a simulated heat wave highlight the importance of understanding oxidative stress and telomere length in reptiles exposed to climate change. Future studies should focus on understanding the mechanisms that regulate telomere length dynamics in the context of heat waves, by assessing, for instance, how heat shock proteins and telomerase reverse transcriptase influence telomere length (Monaghan, 2014). Such information would help predict the physiological capacity to adapt of reptiles at high risk of climate warming.

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Conflict of interest

The authors declare no conflicts of interest.

Appendix A

See Figs. A.1 and A.2

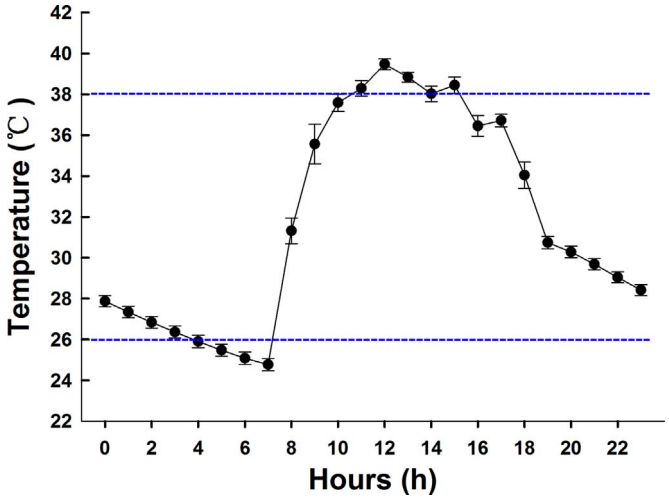

Fig. A.1. Diel variation in the field body temperature of Phrynocephalus przewalskii individuals. The field body temperature (A) of the lizards was monitored in August 2012 and 2013. We designed the thermal regime of the control group based on the field body temperatures of lizards. The blue lines indicate the thermal regime (32 ± 6 °C) used in the control group of this study.

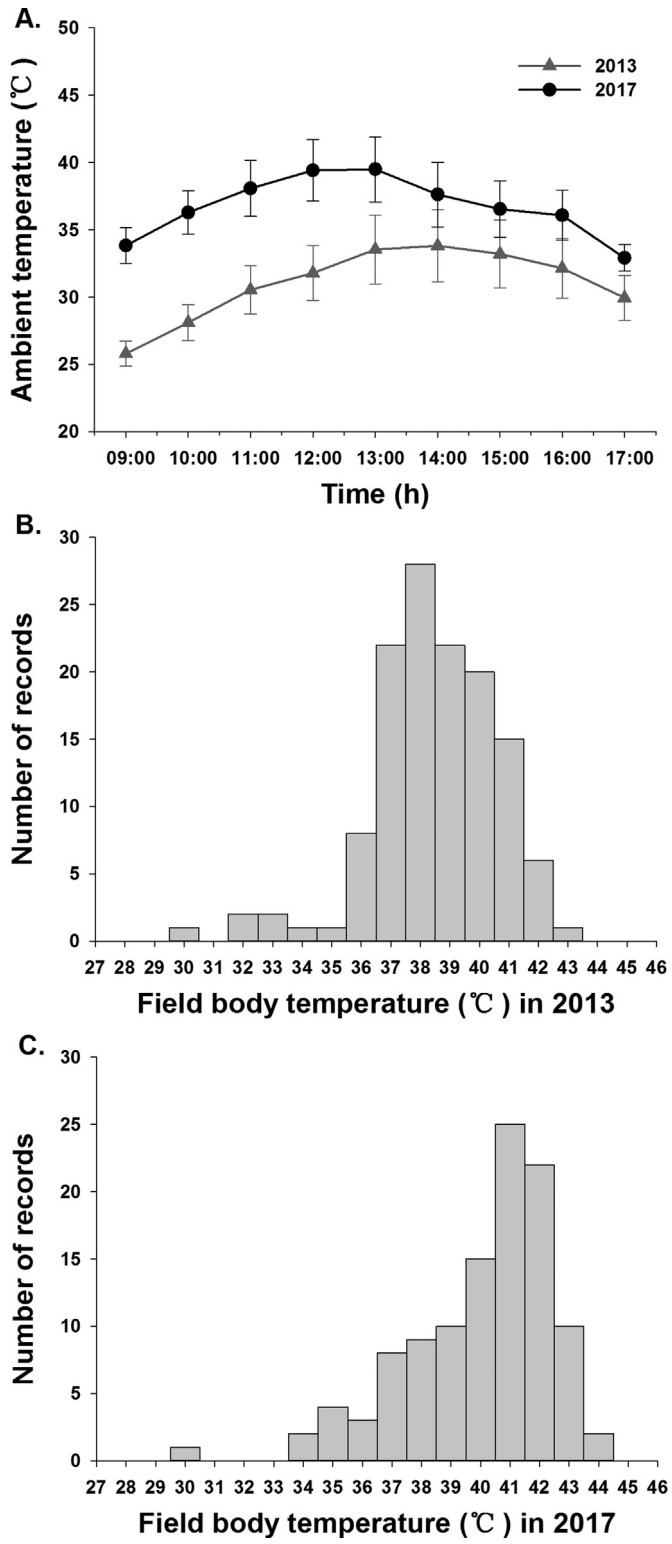

Fig. A.2. Field ambient temperatures (A) and body temperature distribution (B, C) of active lizards (Phrynocephalus przewalskii). In August 2013, and July 2017, we measured body temperatures of adult P. przewalskii and ambient temperatures in our field site where the lizards were collected, which verified that the experimental set-up in our study has incorporated important thermal aspects of the natural environment. We recorded ambient temperatures (air temperatures at 5 cm above the ground) every hour using iButtons (DS1921, MAXIM Integrated Products Ltd., USA). We measured field body temperatures of adult lizards on sunny days. The body temperature of the lizards was measured to the nearest 0.1 ℃ with a UNT325 electronic thermal meter (Shengzhen Meter Instruments, Shengzhen, China) immediately after they were caught.

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